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The culture of cereal rusts in the greenhouse

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The desirability of maintaining cultures of parasitic fungi on the living host in the greenhouse for purposes of study and physiological experimentation is obvious, particularly so with those obligate forms that cannot be cultivated on artificial media. Methods of culture of the powdery mildews of the grasses and other members of the Erysiphaceae, and of a species of *Cystopus*, etc., Peronosporaceae, have been placed on an exact basis by the work of Reed (28, 29) and Melhus (23). No exact data of this nature, however, are available for the rusts. Although a vast number of infection experiments on this group have been made in recent years, these have dealt only incidentally with conditions governing spore germination, infection, and spore formation, and in many cases our knowledge on these points is based on field experiments conducted under conditions not subject to rigid control. There are a great many scattered details in the literature as to conditions affecting the development of the rusts. I shall summarize only those that bear more especially on the problems of growing rusts in the greenhouse.

Smith (33) found that dew is of more importance in determining the prevalence of asparagus rust than rainfall. When little dew is formed infection cannot occur and sporulation may be checked, or altered, with a substitution of the teleuto for the uredo stage. This may occur in midsummer on the vigorously growing host as the result of excessive atmospheric dryness. He states, moreover, that abundance of soil moisture, instead of favoring rust development, acts as a check by giving the host greater vitality. This is in agreement with earlier observations by Stone and Smith (34, 35). They found asparagus beds on light, dry soil heavily rusted while those on heavy, moist soil were comparatively free from rust. Sirrine's (32) observations also support the conclusion that dew is the most important agent in the spread of asparagus rust.

Morgenthaler (25) finds that mechanical injury of the leaves of the host favors teleuto as compared with uredo formation in the case of *Uromyces Veratri.* Tischler (36) found that shoots of *Euphorbia Cyparissias* infected with *Uromyces Pisi* became free from the rust mycelium when grown in a warm (25–27° C.) greenhouse.

Many factors doubtless influence the germination of rust spores. They frequently germinate in a few hours after being placed in water, as noted by de Bary. Often, however, though collected fresh, they fail to germinate for no apparent reason. This "capricious" germination has been noted by a large number of careful observers, and Eriksson, because of this uncertainty, does not consider the aecidiospores of the rusts of the cereals important factors in their dissemination. Schaffnit (31) explains this "capricious" germination on the ground that unless the spores are mature internally before detachment from their stalks they are incapable of germination. Complete maturity is attained only at a sufficiently high temperature (20°–25°) in an atmosphere calm enough to prevent their premature detachment. These conditions are not always realized in nature, hence the lack of uniformity in the results of germination tests.

Freeman (13) and Klebahn (19) both have found that spores which germinate poorly in water may produce an abundant infection on the host, and therefore argue that germination tests are not conclusive unless conducted on the host. Klebahn states that aecidiospores of *Peridermium Strobi* germinated slightly or not at all in water, very vigorously on the *Ribes* host and less vigorously, but abundantly, on a decoction of *Ribes.* Sappin-Trouffy (30) likewise noted a marked difference in the germination of aecidiospores of *Coleosporium Senecionis* in water and in a decoction of *Senecio vulgaris.* Schaffnit (31) on the other hand, obtained no increase in host decoctions over the germination in water nor any effect attributable to a mechanical excitation by the substratum. Marshall Ward (37), was unable to find any effect of raw or cooked extracts of various bromes on the germination of the uredospores of *Puccinia dispersa.*

The effect of various chemicals on rust spore germination has been investigated by Wüthrich (40) and Carleton (6). Wüthrich (see TABLE I) has determined the inhibiting action of different concentrations of various acids and salts on the germination of aecidio- and uredospores of *P. graminis.* Carleton finds that compounds containing Hg, Cu, Fe, Pb, Cr, and strong acids inhibit the germination of uredospores of *Puccinia rubigo-vera*, *P. graminis* and *P. coronata* and that those which contain O, Na, K, Mg, S, C, and NH_3 in large proportions are favorable to germination.

The effect of temperature on spore germination has also received attention. Eriksson (9) found that aecidiospores of *Aecidium Berberidis*, *A. Rhamni*, and *Peridermium Strobi* and uredospores of *Puccinia glumarum*, *P. graminis* and *P. coronata* often germinated more readily at a few degrees below zero C. and on melting ice than at higher temperatures. Uredospores of *P. dispersa* according to Marshall Ward (37) germinate after freezing in ice for ten minutes. He attributes any increase in vigor obtained in this way to the drying action of the freezing and not to the low temperature. They germinated also at 27° but failed to do so at 30° and were killed at 65° to 70°. The optimum is near 20°. They germinate readily, if the spores are properly ripened and fresh and the temperature does not rise above 25°, in light, darkness, or red light, but less readily in blue light. Gibson (14) reports tests with uredospores of *Puccinia Chrysanthemi* as follows: Fifty per cent germination at 6°–6.5°, free germination between 7° and 21°, all at 21°–25°, one eighth at 24°–25°, and none at 30°. If kept dry at 35° for eighteen hours and then removed to 17° they germinate freely. Johnson (18) has determined the minimum, optimum, and maximum temperatures for germination of uredospores of the cereal rusts. These are: for *Puccinia graminis* on wheat, oats, and barley, 2° to 31°; for *P. rubigo-vera* on rye, 2°–30°; and for *P. coronata* on oats, 7°–8° to 30°. The optimum was determined by the general vigor of the germination tube and for all forms studied lies between 12° and 17°. This is somewhat lower than for *P. dispersa* as determined by Marshall Ward. Johnson suggests that these low cardinal temperatures may explain the difficulty of obtaining infections in very warm greenhouses and on hot summer days and may account for the observation that rust epidemics are favored by subnormal temperatures at critical infection periods.

The length of time during which uredospores of *P. graminis* retain their vitality was found by de Bary (3) to vary between one and two months, while the aecidiospores of the same form lost their color and capacity for germination in one month. Marshall Ward (38) obtained germination with uredospores of *P. dispersa* after having kept them in a dry state for 61 days. Miss Gibson (14) reports a germination of 25 per cent. with uredospores of *P. Chrysanthemi* after storage of 71 days but none a week later. One aecidiospore of a sowing of *Phragmidium Rosae-alpinae* germinated after storage of 82 days. Barclay (2) found uredospores of some forms capable of germination during periods of from two to eight months (see TABLE I) in the Himalayas. Bolley (5) obtained a 5 per cent germination with uredospores of *P. graminis* after exposure to air and sunlight during the month of August.

Many observers have shown that various rusts are able to winter over in the uredo stage in some regions, but these data do not involve the determination of the actual time during which the spores are viable.

Gibson (14) has shown that a large number of uredospores will germinate on the leaves of the wrong host and that their germ tubes will enter the stomata without, however, producing an infection. In these cases the end of the germ tube dries up in the substomatal chamber without further development. Because inoculation does not always result in infection, Marshall Ward (37) would distinguish sharply between these terms which are often used interchangeably. The passage of the germ tube into the host should be spoken of as inoculation and the subsequent development in the tissue of the host as infection. Pole Evans (11) has investigated the entrance of the germ tubes of uredospores of *P. graminis*, *P. glumarum*, *P. simplex*, and *P. coronifera* into the stomata of their respective hosts and the establishment of the mycelium in the tissue of the host. The uredospores germinate within twenty-four hours and the infection is well established by the third day. When the germ tube reaches a stoma it forms a swelling or appressorium directly over it. A branch from the appressorium next enters the stomatal slit and forms a large vesicle in the substomatal chamber into which the contents of the appressorium and germ tube are poured. One or more infecting hyphae are now sent off from the substomatal vesicle and these immediately establish connections with the surrounding host cells by means of haustoria. The early stages of development and the position and shape of the substomatal vesicle, and the number of infecting hyphae arising from it, are morphological distinctions between the different species.

In the rusts the period between inoculation and sporulation is known as the incubation period. This apparently varies somewhat with different species but the normal range for the uredo as reported by a number of authors lies between eight and twelve days. Marshall Ward has noted that the normal incubation period is shortened during clear sunny weather and Iwanoff (16) found that shading delayed aecidium-formation in *P. graminis.*

What stimulus, or stimuli, determine the entrance of the germ tube into the stomata of the leaf has not been established. It is perhaps most generally held that the host exerts a chemical influence on the germ tube. If this is true it is apparently not a specific influence, since germ tubes have been shown to enter the stomata of quite the wrong host. Massee (22) endeavored to demonstrate a positive chemotropism but was unable to eliminate the effects of hydrotropism from the experiment. Marshall Ward (37) has noted an apparent heliotropic curvature in the germ tubes of *P. dispersa.* Balls (1) placed uredospores on a rubber film provided with small holes. Laboratory air was on one side of the film while the air on the other side was saturated with moisture at 23°. The germ tubes entered the holes and grew through into the region of higher pressure of water vapor. He believes that growth towards greater moisture will explain the entrance of the germ tube into the host.

A recent article by Melhus (24) on the culture of parasitic fungi deals with the culture of *P. Helianthi*, *P. coronata*, *P. graminis* and *P. Sorghi.* The methods employed by him consist in spraying the plants to be inoculated with a spore suspension, covering with a hood and placing in a refrigerator or humidity-box for twenty-four hours, at 14° for *P. Helianthi*, 16° for *P. coronata*, and 18° for *P. Sorghi.* He finds that *P. coronata* will not maintain itself even though supplied with plenty of host material. It is more difficult to hold in culture than the sunflower rust, a fact which is attributed to the slower growth of the oat plants. Reinoculation is necessary about every three or four weeks. *P. Sorghi* is more easily cultured than the cereal rusts and Melhus has propagated it both winter and summer. The amount of infection increases as the corn plants grow larger and the fungus spreads from one culture to another by natural agencies.

I have tabulated the principal recorded observations on the influence of various conditions on spore germination and development in the rusts in TABLE I. These scattering and somewhat fragmentary records illustrate the incompleteness of our knowledge of rust physiology.

CULTURE METHODS

Preliminary experiments were made to determine how long single infections would maintain themselves under greenhouse conditions and whether they would self-propagate to any extent. The rusts used were *Puccinia dispersa* Erikss. on rye and *Puccinia coronifera* Kleb. on oats. The seedlings were grown in 5-inch pots and infections were secured by atomizing them with a uredospore suspension followed by covering with a bell jar for twenty-four hours. The incubation periods for both forms averaged about twelve days in this preliminary work. The infections secured maintained themselves for about two weeks after the first ripening of the pustules. After this time the number of pustules visibly decreased through withering and dying off of infected leaves and eventually the cultures became entirely free from infection. Close association of non-infected with infected plants did not produce new infections. No marked difference in susceptibility between old and young plants was apparent but the younger were found more desirable for inoculation because their greater compactness facilitated uniform covering with the spray.

My further experiments were planned to meet various requirements. First a method to maintain cultures of as nearly as possible constant virulence, with the fewest necessary transfers, over extended periods of time, was tested out. Such a method is suited, for example, to test the possibility of maintaining the rust for long periods in the uredo stage and for the study of the effects of such conditions of growth on its virulence, incubation period,

TABLE I

Species of rust	Spore form	Author	Conditions influencing spore germination and development									Length of incubation period
			Temperature			Moisture	Chemicals*	Host extracts	Host	Storage	Variation in light	
			Min.	Opt.	Max.		Inhibiting					
							%					
Puccinia graminis	II	DeBary	....	....	....	+ after 2–3 hours in water		......	......	1–2 months	....	8 days
Puccinia graminis	I	DeBary	....	....	....		1. KNO_3 0.5 Na_2CO_3 0.1 $C_2H_2O_4$ 0.1 $C_2H_4O_2$ 0.1 H_2SO_4 0.1 HCl 0.1 $FeSO_4$ 0.01 $ZnSO_4$ 0.01 $ZnCl_2$ 0.01 $CuSO_4$ 0.001 $HgCl_2$	......	......	1 month	....	....
Puccinia graminis	II	Wüthrich		20–21°		+ after 15 hours		......	......	....	....	....
Puccinia graminis	I	Iwanoff	....	....	....	....		......	......	....	Shading lengthens incubation period	....
Puccinia graminis	II	Pole Evans	....	....	....	Dry weather favors development		......	......	....	....	....
Puccinia graminis	II	Eriksson	Low temperatures accelerate			"Capricious"	....	......	......	....	....	....
Puccinia coronata	II	Eriksson	Low temperatures accelerate			"Capricious"		......	......	....	....	....
Puccinia glumarum	II	Eriksson	Low temperatures accelerate			"Capricious"	0.01 H_2SO_4 0.01 $FeSO_4$ 0.001 $ZnSO_4$	......	......	....	....	....

* All data as to chemicals are Wüthrick's.

TABLE I—*Continued*

Species of rust	Spore form	Author	Conditions influencing spore germination and development										Length of incubation period
			Temperature			Moisture	Chemicals		Host extracts	Host	Storage	Variation in light	
			Min.	Opt.	Max.		Favoring	Inhibiting					
Puccinia graminis	I	Wüthrich	......	......	......	....	0.001 $CuSO_4$ 0.0001 $HgCl_2$		......	......	....	....	....
Puccinia graminis	II	Johnson	2°	12–17°	31°	....	......	......	......	......	....	....	....
Puccinia coronata	II	Johnson	7–8°	12–17°	30°	....	......	......	......	......	....	....	....
Puccinia rubigo-vera	II	Johnson	2°	12–17°	30°	....	......	......	......	......	....	....	....
Puccinia rubigo-vera	II	Carleton	......	......	......	....	Na K	Hg Cu	......	......	....	....	....
Puccinia graminis	II	Carleton	......	......	......	....	Mg S	Fe Pb	......	......	....	....	....
Puccinia coronata	II	Carleton	......	......	......	....	Ca NH_3	Cr Alkaloids	......	......	....	....	....
Puccinia coronata	II	Melhus		16°		....	......	......	......	......	....	....	8–12 days
Puccinia Helianthi	II	Melhus		14°		....	......	......	......	......	....	....	10 days
Puccinia Sorghi	II	Melhus		18°		....	......	......	......	......	....	....	8 days
Puccinia rubigo-vera	II	Plowright	+ after exposure to − 5° in open			....	......	......	......	......	....	....	....
Puccinia rubigo-vera	II	Bolley	......	......	......	....	......	......	......	......	30 days in open	....	....
Puccinia dispersa	II	Marshall Ward	10–12°	20°	26–27°	....	......	CO_2	No influence	......	61 days	+ in dark and in red or blue light	7–10 days
Puccinia dispersa	II	Freeman	......	......	......	Often poor in water	......		......	Good	....	....	....
Puccinia Asparagi	I, II	Smith	......	......	......	Dew essential for infection	......	......	......	......	....	....	....

TABLE I—*Continued*

Species of rust	Spore form	Author	Conditions influencing spore germination and development										Length of incubation period
			Temperature			Moisture	Chemicals		Host extracts	Host	Storage	Variation in light	
			Min.	Opt.	Max.		Favoring	Inhibiting					
Phragmidium Rosae-alpinae	I	Gibson	......	......	......	....	......	......	......	Germ tubes enter wrong host	82 days	....	....
Puccinia Chrysanthemi	II	Gibson	6–6.5°	21–25°	25–30°	....	......	......	......	......	71 days	....	....
Puccinia Chrysanthemi	II	Jacky	+ after exposure to − 25° in open			....	......	......	......	......	....	....	....
Phragmidium obtusum	II	Dietel	+ after breaking out of ice and snow			....	......	......	......	......	....	....	....
Peridermium Strobi	I	Klebahn	....			Weak in water	......	......	Fair	A-bundant	5 weeks	....	....
Peridermium Soraueri	I	Klebahn	....			....	......	......	......	......	20 days	....	....
Coleosporium Senecionis	I	Sappin-Trouffy	....			Poor or none in water	......	......	A-bundant	......	....	....	....
Uromyces Pisi	I	Tischler	Host outgrows rust in moist air at 25–27°			....	......	......	......	......	....	....	....
Uredo Bupleuri	II	Barclay	....			....	......	......	......	......	8 mo. 12 d.	....	....
Uredo Gomphrenatis	II	Barclay	....			....	......	......	......	......	7 mo. 7 d.	....	....
Puccinia Prenanthis	......	Barclay	....			....	......	......	......	......	7 mo. 6 d.	....	....
Puccinia Caricis-filicinae	......	Barclay	....			....	......	......	......	......	5 mo. 12 d.	....	....
Uromyces Vossiae	II	Barclay	....			....	......	......	......	......	5 mo.	....	....
Puccinia Acetosae	......	Barclay	....			....	......	......	......	......	3 mo. 17 d.	....	....
Uromyces Pisi	......	Barclay	....			....	......	......	......	......	2 mo. 15 d.	....	....
Melampsora Lini	......	Barclay	....			....	......	......	......	......	2 mo. 15 d.	....	....
Puccinia flosculosorum	......	Barclay	....			....	......	......	......	......	2 mo. 7 d.	....	....

etc. It was found that it was necessary to transfer the infection to new cultures once a month. Five ripe, oval pustules were selected for each inoculation. The spores from these were removed with a scalpel and immersed in 25 c.c. of water in the bottle of the atomizer. This was then shaken vigorously to secure uniform distribution of the spores before application and the culture was subsequently covered with a bell jar for twenty-four hours. "Spring" rye and "Kherson" oats were used exclusively. About twenty-five seeds were sown to a 5-inch pot.

Six transfers at intervals of a month were made. At first seed for the next culture were sown two weeks prior to the date set for transfer, but a further simplification of method was secured by sowing a month prior to inoculation. Thus sowing and transfer could be made at the same time. Plants were a month old when inoculated and two months old when abandoned.

Well-infected cultures on both oats and rye were maintained for six months in this way. No attempts were made to measure exactly the degree of infection secured but the pustules were seemingly as numerous at the end of the period as at the beginning. Germination tests of spores in drop cultures made at various times gave 50–75 per cent. germination in six to twelve hours. The incubation periods during the six months were quite uniform. Twelve days was the longest incubation period recorded and ten days the shortest.

Cultures made in this way show relatively few sori. The method provides for maintenance of a rust culture but it does not provide an abundant supply of infected plants at all times. During the incubation period the pustules have disappeared from the old cultures and have not matured on the new ones. To have abundantly infected plants continuously available the following method was used. Transfers were made once a week instead of once a month. Since two weeks were required for complete ripening of the pustules it was necessary to run alternate series of host cultures. One series was ready for transfer one week and the other series the week following. Seeds for subsequent cultures of each series were sown at the time of inoculation and the seedlings were thus two weeks old when the rust was transferred to them. Cultures of *P. coronifera* have been maintained for eight months by this method and the fungus has gone through thirty-seven generations of the uredo stage with no decrease in virulence.

It was soon found that even more simple methods of inoculation are equally if not more efficacious than the application of spore suspensions with an atomizer. The plants to be inoculated are first thoroughly atomized with water. A well-infected culture pot bearing fully ripe pustules is then held in a horizontal position immediately above them and given a vigorous shaking. The spores that fall from above are caught in the small drops of water provided by the spray. If the culture used is heavily infected the falling spores may be seen as yellow clouds. The inoculated plants are then covered for twenty-four hours. Four pots of seedlings can be inoculated simultaneously from the same culture and a uniform degree of infection secured on all four by placing them close together and holding the culture somewhat higher than for inoculating a single pot. It was found that spraying prior to inoculation could be dispensed with but the pustules secured were somewhat less numerous than when the spray was applied. To secure the best results the culture used for transfer must be heavily infected and the transfer made shortly after the ripening of the pustules. If transfer is delayed more than a week after sporulation begins, inoculation with a spore suspension must be resorted to.

Very numerous and uniformly distributed pustules were secured and maintained by this dry-spore method of inoculation. The approximate number of pustules per plant in a culture was determined in the following way.

For facility in counting the surface area of the pot it was divided into smaller areas with strips of cardboard. The number of plants and infected parts on each of the smaller areas was then easily ascertained. Ten plants were then taken at random from the culture, removed to the stage of a binocular, and the number of pustules on the infected parts determined. TABLE II shows the amount of infection on a typical culture. In this culture all of the first leaves were infected, 62.5 per cent of the second leaves, and 29.1 per cent of the sheaths. The average number of pustules on the first leaves of the ten selected plants was 574.4, on the second leaves, 22.9, and on the sheaths, 1.4. The small number of pustules on the second leaves was due to the fact that only their tips were exposed at the time of inoculation. The number of pustules on the upper and lower surfaces of a leaf is sometimes equal and two pustules are often situated directly opposite each other. The average number of pustules per plant maintained in the mass culture experiments was about 200–300. The lowest average recorded for a culture was 161.6, the largest 598.7. The largest number of pustules counted on a single plant was 996 (plant 3, table II).

Table II.

Analysis of infection on culture 7c

Treatment: Innoculated by dry spore method, covered with bell jar. Number of plants, 72. Date planted, January 1. Date innoculated, January 13. Date of sporulation, January 24.

Number of parts	Part of plants	No. of parts infected	Per cent. infected
72	First leaves	72	100
72	Second leaves	45	62.5
72	Leaf sheaths	21	29.1

Number of pustules on ten plants selected at random

No. of plant	First leaves		Second leaves		Leaf sheaths	Total, entire plants
	Lower surface	Upper surface	Lower surface	Upper surface		
1	297	246	27	30	1	601
2	215	341	12	21	5	594
3	506	441	18	31	0	996
4	444	521	7	11	3	986
5	234	336	4	5	0	579
6	225	249	0	0	0	474
7	376	403	10	13	3	805
8	99	140	0	0	2	241
9	53	115	1	3	0	172
10	189	314	14	22	0	539
Total....	2638	3106	93	136	14	5987
Ave......	263.8	310.6	9.3	13.6	1.4	598.7
Ave......	574.4		22.9		1.4	598.7

Normal development of the uredosori of P. coronifera

The period of incubation for the rust in the open greenhouse varied between eight and eleven days from October to December. Twelve days was the constant incubation period during December but this decreased to nine days in the latter part of January and remained at nine days throughout February and March. The sequence of stages during a nine days incubation period may be divided into two periods. First, a period of vegetative development during which no evidence of infection is seen. Second, a fruiting period during which the stages in the formation of the pustules are apparent. The first period occupies five days after inoculation and the first visible evidences of pustule formation become apparent on the sixth day. The leaves on this day have a faint mottled appearance which is due to the presence of small areas that are lighter in color than the surrounding leaf tissue. These areas are visible only by transmitted light. On the seventh day the light areas become more conspicuous and their boundaries more sharply defined. The areas become slightly swollen on the eighth day and a light orange color is apparent. During the next twenty-four hours the development of the pustules is rapid. They continue to swell until the epidermis of the leaf is ruptured by a longitudinal slit and the orange mass of spores is extruded. The mass of spores hangs together for a time but breaks apart on drying and falls from the leaf as separate spores or in small groups when the leaf is disturbed.

OBSERVATIONS ON CONDITIONS AFFECTING SPORE GERMINATION, INFECTION, AND RATE OF DEVELOPMENT

Effect of moisture

It has been demonstrated a number of times during this culture work that a humid atmosphere provided by covering with a bell jar is necessary to secure infection. Abundant moisture may be supplied at the time of inoculation and still the plants will not become infected, in the greenhouse, unless they are covered soon afterwards. The drops of water apparently dry up before germination and infection result. The per cent of saturation in the greenhouse in which my experiments were made as obtained from the hygrometer records, averages about 75–80 per cent with a temperature range between 55 per cent. and 85 per cent. Although the conidia of *Erysiphe graminis* infect the cereals spontaneously under these conditions, the uredospores of *P. coronifera* will not do so.

To test the possibility of providing conditions of humidity under which the rust might become self-propagating by close association of cultures, a sash frame culture box, 3 ft. square, was made of five window sash. The humidity maintained here when cultures were growing in it was quite constant and averaged 93 per cent. with occasional fluctuations of 2–3 per cent. Even in such a humid atmosphere new infections occurred only sparingly, although cultures were sprayed and heavily inoculated. To obtain good infections it was necessary to cover the cultures with bell jars as in the open greenhouse.

A direct comparison of the effects of covering and not covering in the culture box was obtained by a statistical study. Two pots of seedlings, each seven days old, were inoculated simultaneously from the same culture, after which one was covered for twenty-four hours and the other was left uncovered in the culture box. During the remaining eight days of the incubation period they were exposed to equal conditions of humidity. The difference in the degree of infection obtained on the two cultures was marked. The average number of pustules per plant on the covered culture was 161.6 as compared with 10.4 on the non-covered. This would be but a 6 per cent infection on the non-covered culture if that obtained on the covered is regarded as the normal. The difference in the degree of humidity to which the cultures were exposed for twenty-four hours after inoculation could not have been more than 7 per cent, as the average in the culture box was 93 per cent and the atmosphere under the bell jar was presumably saturated. It is rather striking that this difference of 7 per cent should have produced a difference in degree of infection of 94 per cent. The spores that produced the 6 per cent normal infection on the non-covered culture probably germinated more rapidly or were more favorably located with reference to moisture than the bulk of the spores.

Effect of temperature

To test the effect of different temperatures on the degree and rate of development of *P. coronifera*, two cultures of the same age were inoculated simultaneously from the same stock culture. Immediately afterwards one was placed in the greenhouse "stove" where the temperature ranges between 20° and 30°.* The temperature here was quite constant for each twenty-four hours throughout the experiment. It did not fall below 25° from 10 A.M. to 5 P.M. nor rise above 20° from midnight to 6 A.M. The other culture was placed in the greenhouse where the temperature fluctuation was between 14.5° and 21°. Here the temperature remained quite constant at 16° for the greater part of the twenty-four hours. It reached 21° for a short period at noon and 14.5° at midnight. Both cultures were covered after inoculation. The first visible signs of infection became apparent on the culture in the "stove" on the fourth day after inoculation and fully ripe pustules were produced on the seventh day. Evidences of infection did not become visible on the culture in the greenhouse until the seventh day after inoculation, which was the date of sporulation for the culture in the "stove," and ripe pustules were not formed until the twelfth day. The high temperature of the "stove," which was an average increase of about 7–8°, was apparently responsible for a marked increase in the rate of development of the fungus and a decrease of five days in the incubation period. No differences in the degree of infection secured on the two cultures were apparent. The experiment was repeated immediately, but without a control, and the incubation period for this culture in the "stove" was but six days. This is the shortest incubation period I have observed.

Further cultures gave results as follows. No. 7 in the "stove," incubation period seven days. The temperature for each day was 20° from 6 P.M. to 6 A.M. and 25–30° from 8 A.M. to 2 P.M. Three controls in the greenhouse, incubation period nine days. Temperature 10–15.5° from 6 P.M. to 6 A.M. and 15.5–21° from 9 A.M. to 5 P.M. No. 8 in the "stove," incubation period nine days. There were many minor fluctuations in this period. The maximum was 30°, the minimum 8°. The general range was distinctly lower than in the preceding test. Two controls in the greenhouse, incubation period twelve days. Temperature 10–15.5° from 6 P.M. to 6 A.M. and 15.5–21° from 10 A.M. to 3 P.M.

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* The temperature records were obtained with Richard Frères' self-recording thermometers.

Effect of light

To determine the effect of light exclusion on spore germination and rate of development, four culture pots of the same age, seven days, were inoculated simultaneously. Immediately afterwards one of the four was transferred to a physiological dark room which joins the greenhouse. A continuous circulation of air between the two rooms is maintained by an electric fan. Thus the average degree of humidity of the dark room, which is about 80 per cent., does not fall below that of the greenhouse although the range of fluctuation, which is from 60 per cent. to 95 per cent., is somewhat greater. The other three cultures were placed in the culture box as controls. The culture was exposed in the dark room for three days and at the end of this time was returned to the culture box. The plants at this time were quite as green and fresh as those of the control cultures and could not be distinguished from them. The incubation period for the three controls was eight days, while that of the culture left three days in the dark room was eleven days. At the time of sporulation some of the leaves of this culture showed signs of yellowing at their tips. No pustules were produced on these discolored areas but on the normal green parts they were as numerous as on the controls. The difference of three days in the incubation periods is exactly equal to the period of light exclusion and indicates a complete arrest of the development of the fungus in the dark room.

The effect of light exclusion during the latter part of the incubation period was also tested. Four cultures were inoculated and placed in the culture box. Four days later one of them was transferred to the dark room, where it was left four days, and then returned to the culture box. No signs of infection were visible on it at this time, while the unripe pustules on the controls were plainly visible. The pustules on the controls ripened on the ninth day, while three additional days, twelve days in all, were necessary for a similar development of the culture that had been in the dark room. By excluding light four days in the latter part of the normal incubation period, the maturation of the rust had been delayed three days. This shows that even after the fungus has become well established in the host its development is strongly retarded in complete darkness.

The average difference in degree of humidity, of approximately 13 per cent, between the culture box and the dark room could not have been an important modifying factor in these results, as I have found that the incubation period is not modified by relative degrees of humidity after the first twenty-four hours. Thus parallel sets of cultures, when grown in the culture box and in the greenhouse under similar conditions of light and temperature but with the same difference in humidity that is found between the culture box and the dark room, had the same incubation period. Likewise when a culture was kept under a bell jar during the entire incubation period, with occasional removal for change of air, there was no difference between its incubation period and that of the control in the greenhouse that was covered for the first twenty-four hours only.

The question naturally arises how this retardation of the growth of the fungus is brought about. It may be the direct effect of total absence of light on the fungus itself. Then, again, it is possible that the fungus simply suffers from lack of food, since the host is incapable of assimilation in the darkness. It seems hardly possible, however, that such a complete inhibition in the growth of the fungus should have resulted in the brief time involved unless it is dependent on the transition products in photosynthesis. This latter possibility is by no means inconceivable and should this explanation prove the correct one it could be made the basis for an explanation of the obligate parasitism of the rusts and their inability to develop on any form of artificial medium.

VIABLE PERIOD OF UREDOSPORES

Two series of tests were made to determine the period through which the uredospores of *P. coronifera* would retain their vitality. Ripe spores were removed from pustules on the cultures and placed in small gelatine capsules which were then stored in the laboratory at room temperature. The first set of spores was stored on March 13. A drop culture made on this date gave a high per cent of germination in twelve hours. Drop cultures of the stored spores were made on March 19, 23, and 28 and April 12 and 30. From 9 to 16 per cent. of the spores sown germinated in each of these tests. No further trials were made until June 1 and at this time the spores were colorless and failed to germinate. The last germination obtained was after 48 days of storage and the spores had lost their capacity for germination at some time between 48 and 80 days.

Another lot of spores were stored on November 26. These germinated in gradually decreasing per cents on January 8, 18, and February 2, 11, 18. The length of time required for the development of a germination tube increased from a period of 6–12 hours in November to a period of 36–48 hours in February. In the last drop culture made, on February 18, only 29 spores of 1,013 tested germinated. These few spores, about 0.2 per cent, had germinated after 84 days storage.

CONTROL OF MILDEW

The powdery mildew, *Erysiphe graminis*, became so abundant on the oats cultures that measures for its control became necessary. The mildew spreads rapidly in the greenhouse and outgrows the rust to such an extent that rust culture work may be seriously interfered with. Attempts at exclusion of the mildew by isolation of the cultures and inoculation with uredospores selected from apparently non-mildewed leaves proved unavailing.

The control and total exclusion of the mildew was achieved by treatment with sulphur dust. This was applied with a powder gun, twenty-four hours after rust inoculation. The plants were also atomized with a weak solution of sulphuric acid (1/1000) prior to the application of sulphur and were covered afterwards for twenty-four hours. With this treatment no mildew developed while control cultures became heavily infected.

This work was undertaken at the suggestion of Prof. R. A. Harper, to whom I am indebted for suggestions and criticisms.

SUMMARY

1. Two of the cereal rusts, *Puccinia dispersa* Erikss., on rye, and *P. coronifera* Kleb., on oats, have been cultured in the uredo stage, on the living hosts in the greenhouse, for a consecutive period of six months, from December 1912 to June 1913, by the transfer of infection once a month. *P. coronifera* was also cultured for a period of eight months, from September 1912 to May 1913,

with transfer of infection once a week. During this period the rust went through 37 generations of the uredo stage. No decrease in the degree of infection secured resulted from such continuous culture.

2. The average degree of infection maintained in mass cultures was approximately 200 pustules per plant. The largest number of pustules counted on an individual plant was 996.

3. *P. coronifera* does not self-propagate to any extent even when abundant host material is supplied and a constant humidity of 93 per cent is maintained.

4. High humidity is the essential factor in securing successful inoculation with uredospores of *P. coronifera*. No infections resulted when cultures were exposed in an atmosphere of 75 to 80 per cent of humidity, and at 93 per cent only 6 per cent of the normal degree of infection was obtained. Normal infections were secured only when cultures were covered with a bell jar for twenty-four hours subsequent to the application of spores.

5. The rate of development of *P. coronifera* increased with temperature increase. A decrease in the normal incubation period of five days, or 41 per cent, was produced in the "stove" where the temperature ranged from 20° to 30° while the range at which the normal cultures were grown was 14.5° to 21°.

6. Total light exclusion either early or late in the incubation period checks the development of *P. coronifera* and results in an almost complete cessation of growth.

7. Uredospores of *P. coronifera* when stored at room temperature gradually lose their capacity for germination. A 0.2 per cent germination was obtained after storage of eighty-four days.

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